



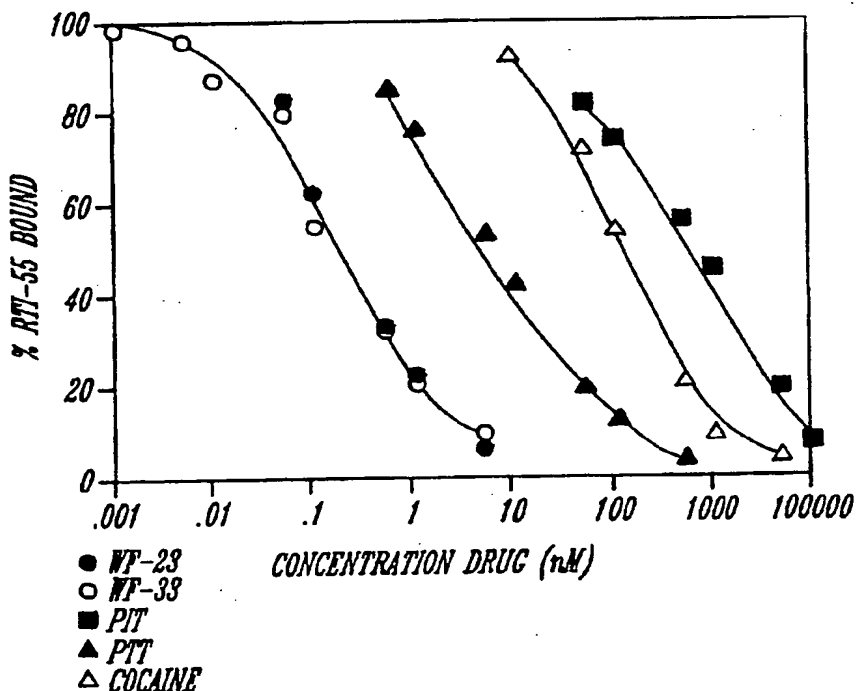
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(54) Title: TREATMENT PROCESS WITH BIOLOGICALLY ACTIVE TROPANE DERIVATIVES

(57) Abstract

Selective blockade of DA and 5-HT uptake sites with 3-aryltropine derivatives.



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**Title: TREATMENT PROCESS WITH BIOLOGICALLY ACTIVE
TROPANE DERIVATIVES**

GRANT REFERENCE

This invention was made with government support under R01-DA-6301-02 and P50-DA06634 awarded by the National Institute on Drug Abuse. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The tropane skeleton is a basic structural unit that can lead to compounds with diverse Central Nervous System (CNS) activity. Due to the rigid nature of the structure, the possibility exists for the preparation of highly selective compounds. This application describes the synthesis of tropane derivatives that selectively bind to monoamine neurotransmitters and thus have the potential for the treatment of major depression, Parkinson's disease and attention-deficit hyperactivity disorder (ADD).

Two important central nervous system neurotransmitters are serotonin (5-HT) and dopamine (DA). Together with norepinephrine and epinephrine, these neurotransmitters comprise the group of agents known as the monoamines. Either 5-HT or DA have been implicated in a variety of disorders, including depression, Parkinsons disease, ADD, obesity and cocaine addiction.

Major depression represents one of the most common mental illness, affecting between 5-10% of the population. The disease is characterized by extreme changes in mood which may also be associated with psychoses. It has generally been found that most

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antidepressant agents exert significant effects on the regulation of monoamine neurotransmitters, including DA, 5-HT and norepinephrine. The tricyclic antidepressants, such as imipramine, are the most commonly used drugs for the treatment of depression. Their ability to inhibit the neuronal uptake of norepinephrine is believed to be a major factor behind their efficacy.

A number of new types of antidepressants have been developed in recent years. Two such compounds that are marketed in the U.S. are trazodone and fluoxetine. Both of these compounds interact with the regulation of 5-HT. Trazodone potentiates the actions of 5-HT while fluoxetine is a potent and selective inhibitor of 5-HT reuptake. 3-Chloroimipramine which inhibits both 5-HT and norepinephrine reuptake has been extensively used as an antidepressant in Europe and Canada. Other compounds which are of current interest or have been examined as antidepressants include fluvoxamine, citalopram, zimeldine, bupropion and nomifensine. All of these drugs inhibit monoamine uptake mechanisms, but differ in selectivity between the dopamine, 5-HT and norepinephrine transporters.

Other syndromes also respond to antidepressant drugs. These include (1) severe anxiety syndromes characterized by panic reactions, and (2) obsessive-compulsive disorder, both of which seem most likely to respond to 5-HT selective agents. Monoamine uptake blockers have also been useful in treatment of chronic pain, neuralgias, migraine, sleep apnea, fibromyalgia, and irritable bowel syndrome.

Parkinson's disease affects about 1% of the population over the age of 65 and leads to serious

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neurological disorders. The main clinical features of the disease are centered around disruption of motor function, such as walking, speech, eating and other skilled acts. It has been recognized that the disease is the result of dopamine deficiency in the basal ganglia. Thus, drugs that can increase the levels of dopamine have the potential to be effective medications for the treatment of Parkinson's disease. The most effective drug in this regard has been levodopa which acts as a biogenic precursor to dopamine.

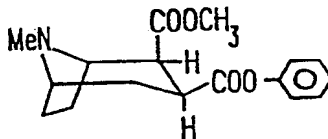
Considerable attention has recently been directed to the condition known as attention-deficit hyperactivity disorder. Children with this condition tend to be very active physically but have great difficulty with situations requiring long periods of attention. Consequently, they tend to underachieve academically and can be very disruptive. Furthermore, these behavioral problems often persist in modified forms into adulthood. The condition appears to be associated to the effect of monoamines in the cerebral cortex, which are involved with control of attention. A number of stimulant drugs such as dextroamphetamine, methylphenidate as well as the tricyclic antidepressants, antipsychotic agents and clonidine have been used as medications to control the disorder. Many of these drugs interact with the monoamine uptake transporters.

Another disorder for which inhibitors of monoamine transport are useful therapeutic agents is obesity. In general, sympathomimetic drugs (i.e., those which increase synaptic levels of monoamines) promote weight loss by suppressing appetite. Drugs

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like mazindol, which act as sympathomimetic agents by blocking monoamine uptake, have been useful in the treatment of obesity.

Cocaine has the following formula:



The basic ring structure of cocaine is a tropane ring system.

It has previously been shown that cocaine and related compounds are potent inhibitors of dopamine reuptake and this may lead to compounds with reinforcing properties. In recent years a number of new extremely potent cocaine analogs have been prepared based on the tropane structure (Abraham et al., *Journal of Medicinal Chemistry* 1992, 35, 141; Boja et al., *European Journal of Pharmacology*, 1990, 183,329; Boja et al., *European Journal of Pharmacology*, 1991, 194, 133; Carroll et al., *Journal of Medicinal Chemistry*, 1992, 35, 969; Carroll et al., *Journal of Medicinal Chemistry*, 1992, 35, 1813; Carroll et al., *Journal of Medicinal Chemistry*, 1992, 35, 2497; Cline et al., *Journal of Pharmacology and Experimental Therapeutics*, 1992, 260, 1174; Cline et al., *Synapse*, 1992 12, 37; Kozikowski et al., *Medicinal Chemistry Research*, 1991, 1, 312; Kozikowski et al., *Journal of Medicinal Chemistry*, 1992, 35, 4764; Lewin et al., *Journal of Medicinal Chemistry*, 1992, 35, 135; Madras et al., *Molecular Pharmacology*, 1989, 36, 518). All of these compounds are based on the tropane skeleton and tend to selectively bind to

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the dopamine transporter. Certain structural variations can lead to compounds that bind with very high selectivity to the dopamine reuptake site (Carroll et al, *Journal of Medicinal Chemistry*, 1992, 35, 2497). However, all of these tropane derivatives are very similar to each other because they are all derived from cocaine as starting material.

It has now been discovered that if the tropane ring system is modified, particularly at the aryl moiety as hereinafter described, compounds can be produced which are more selective in binding to 5-HT transporters as compared to DA transporters. Since these modified tropanes (as described below) bind preferentially to the 5-HT transporter, they may preferentially block 5-HT transport, thus, increasing synaptic levels of 5-HT. This may be helpful in treating diseases related to 5-HT function. Similarly, tropane analogs can be synthesized which selectively block DA transporters and selectively increase synaptic levels of DA.

In principle, the tropane skeleton is ideally suited to prepare highly selective compounds because it is a rigid structure and tropane derivatives will have rather limited conformational flexibility. Such derivatives may be altered by appropriate structural changes so that analogs favoring binding to either the 5-HT or DA reuptake site could be prepared. The novel chemistry that has been developed, as referred to in our parent application, has enabled preparation of a much wider range of tropane analogs than was previously accessible, leading to novel structures with selective biological activity.

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Accordingly, it is a primary objective of the present invention to provide a process for preparing tropane analogs which are selective inhibitors of either 5-HT or DA reuptake.

Another primary objective of the present invention is to prepare a range of tropane analogs which can be investigated as drugs for the treatment of chronic depression.

A still further objective of the present invention is to provide a wide range of tropane derivatives which can be systematically used and tested to determine structure-activity relationships for binding at dopamine, 5-HT and norepinephrine transporters.

A further objective is to provide a treatment system for diseases whose course can be altered by patient treatment with compounds that selectively bind to either the 5-HT or DA reuptake site and therefore prevent neurotransmissions at this site.

SUMMARY OF THE INVENTION

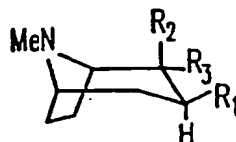
Biologically active derivatives of the tropane ring system are provided which selectively bind either to the 5-HT or DA reuptake site, leading to compounds which have use for treatment of clinical depression, Parkinson's Disease, ADD and obesity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 2, 3, & 4, show the potencies of various analogs of the present invention 5-HT and DA in binding to transporters. These results demonstrate analogs with three different categories of selectivity: DA-selective, 5-HT selective and non-selective.

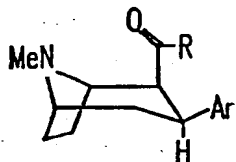
DETAILED DESCRIPTION OF THE INVENTION

The focus of this application will be on uses of tropane derivatives of the general formula:



Wherein R_1 is an aromatic moiety and may be any 1-naphthyl, 2-naphthyl, phenyl, C_1 to C_8 alkylaryl or indole moiety. Preferred are isopropylphenyl and naphthyl. R_2 and R_3 may be as follows: Only one of R_2 and R_3 can be hydrogen at the same time and each of R_2 and R_3 can be a ketone moiety, $(\overset{\text{O}}{\parallel}\text{C}-R_{2-3})$ an ester moiety, $(\overset{\text{O}}{\parallel}\text{C}-OR_{2-3})$ a phosphonate, a sulfone moiety, a cyano, an oxazole, or an imidazole. It is preferred that R_2 and R_3 be selected from ketone moieties or ester moieties, preferably C_1 to C_8 alkyl or alkoxy. If desired the M_e group may be more generally described as R_4 which may be hydrogen or lower (C_1 to C_8) alkyl.

The very most preferred compounds for use in the present process are:



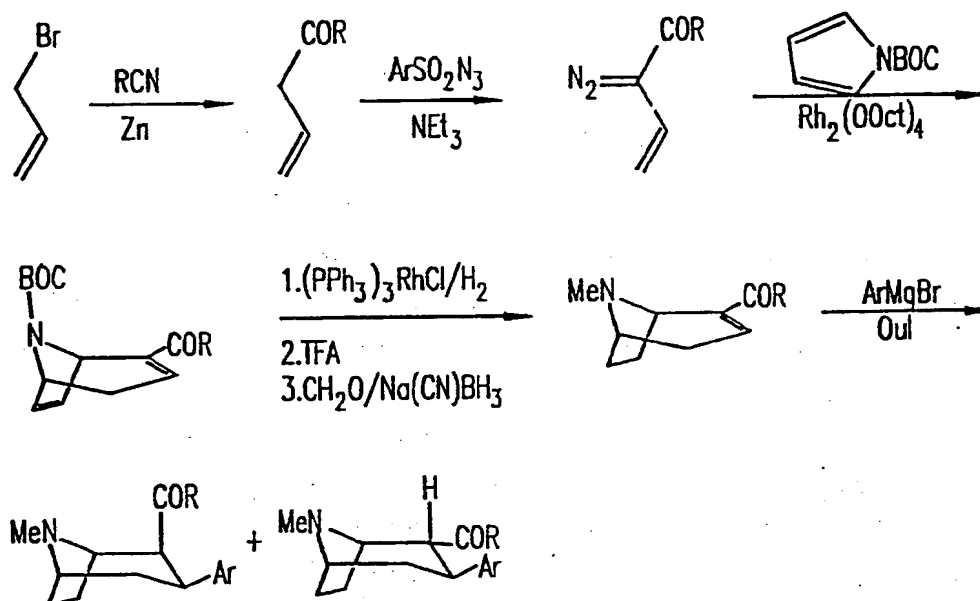
Wherein R is C_1 to C_8 and Ar is an aryl moiety as earlier defined.

The synthesis of the tropane derivatives was achieved by the general scheme shown below. The experimental procedure for the final step has been

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described in detail in the original patent. The details of the earlier steps have been reported (Davies, et al., *Journal of Organic Chemistry*, 1991, 56, 5696).



Basically, in the process of that case, 3-aryltropane derivatives are prepared by reacting 8-azabicyclo[3.2.1]oct-2-ene with an aryl Grignard reagent in the presence of catalytically effective amounts of copper (I) and/or copper (II) salts. The 3-aryl-tropane derivative starting material can be conveniently prepared by decomposing functionalized vinyl diazomethanes in the presence of certain pyrroles, preferably in substantial excess of the stoichiometric amount, using a decomposition catalyst, preferably a rhodium catalyst. The catalyst may also be a copper, palladium or silver salt catalyst. This provides a bicyclic intermediate containing the basic

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tropane ring system which is thereafter converted to an 8-azabicyclo [3.2.1]oct-2-ene, which itself may be used as a starting material to react with an aryl Grignard reagent in providing the synthesis route to the unique cocaine analogs of the present invention.

The starting material of the process is, namely the 8-azabicyclo[3.2.1]oct-2-ene, and has the following formula:



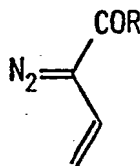
In the above formula R is selected from the group consisting of C₁ to C₈ alkyl and C₁ to C₈ oxyalkyl. In other words, the two position moiety may be functionally substituted by ketone groups or ester groups.

One of the present inventors, namely Dr. Huw M. L. Davies, has previously published concerning the general synthesis used for the starting material of the parent case, namely synthesizing 8-azabicyclo [3.2.1]oct-2-ene of the above formula. In this regard see, Davies, et al., "Novel Entry to the Tropane System by Reaction of Rhodium (II) Acetate Stabilized Vinylcarbenoides with Pyrroles," Tetrahedron Letters, vol. 30, no.35, pp. 4653-4656, (1989) a December 1990 abstract of a regional ACS meeting held in New Orleans, entitled Davies, et al., "Chemistry of Vinylcarbenoids with a Single Electron Withdrawing Group, an Approach to Tropane Alkaloids", American Chemical Society, Dec. 5-7, 1990, pp. 181-182; Davies, et al., "Synthesis of \pm Ferruginine and Anhydro-ecgonine Methyl Ester by a Tandem Cyclopropanation/

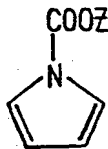
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Cope Rearrangement", Journal of Organic Chemistry, 1991, Vol. 56, pp. 5696-5700. The subject matter of each of these publications of Davies et al is incorporated herein by reference and therefore need not be described in full detail. However, certain preferred process operations, not specifically mentioned in the above articles, are described herein for sake of completeness.

Preparation of the starting material for the Grignard addition of the present invention, namely, preparation of 8-azabicyclo[3.2.1]oct-2-ene as above described employs in its first step a process of decomposing of a functionalized vinyl diazomethane of the formula:



in the presence of at least a stoichiometric amount of a pyrrole of the formula:



wherein Z is a functional group protector, and also in the presence of a small but effective amount of a decomposition catalyst selected from the group consisting of rhodium, copper, palladium and silver salts, to provide an intermediate bicyclic compound.

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R as shown above represents a C₁ to C₈ alkyl or C₁ to C₈ oxyalkyl. Preferably R is an alkyl and therefore as explained herein after, the resulting analog of cocaine ultimately prepared will have a ketone group at the two position. In the pyrrole, Z represents a functional group protector such as trimethylsilylethyl, although it is understood that other classic protecting groups such as tertiarybutyl group may also be employed.

The amount of the pyrrole for this first reaction scheme needs to be at least a stoichiometric amount in comparison with the vinyl diazomethane and preferably is in excess of the stoichiometric amount, perhaps within the range of a two-fold to a five-fold excess. An excess is preferred in terms of achieving the desired high yields of the bicyclic intermediate because the vinyl diazomethane is decomposed to a very reactive intermediate, namely a vinylcarbenoid which will, unless it is trapped by use of stoichiometric excesses of the pyrrole, rapidly decompose.

The pyrroles above described can be conventionally prepared using well known chemistry as described in the Journal of Organic Chemistry, 1991, vol. 56 article, of the author earlier cited. The reaction is preferably run at a temperature of within the range of from 25°C to about 100°C, preferably at about 80°C. The reaction can be run at 25°C if there is slow addition of the vinyl diazomethane to the pyrrole. The pressure is not critical in this reaction step.

As explained above, the reaction is conducted in the presence of a decomposition catalyst selected from the group consisting of rhodium, copper, palladium and silver salts. Preferably the catalyst is a rhodium

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salt catalyst and may be a rhodium (II) acetate, mandelate, trifluoroacetate, hexanoate, pivalate or octanoate. The presently most preferred catalyst is rhodium octanoate which seems to allow higher yields of desired product. The amount of catalyst may vary from 0.25 mole per cent to about 2.0 mole per cent of the vinylldiazomethane, and is preferably about 1.0 mole per cent of the amount of the vinylldiazomethane reactant.

Reaction time does not appear to be critical and the time may vary from a few minutes up to several hours if drop wise addition is accomplished. The other carbon atoms of the 8-azabicyclo[3.2.1]oct-2-ene can include substituents other than hydrogen (e.g. one or more of the other carbon atoms of the bicyclic system can include a lower alkyl substituent group) because a more highly substituted pyrrole or vinylldiazomethane may be used as starting material.

This first step reaction produces an intermediate bicyclic compound which upon hydrogenating, removal of the deprotective group and reductive methylation is converted to the earlier described 8-azabicyclo[3.2.1]oct-2-ene. The hydrogenation, deprotecting and reductive methylation are all well known steps and need not be described herein.

Where R equals methyl and the protecting group used is trimethylsilyl the intermediate is methyl 8-(2-(trimethyl-silyl)ethoxycarbonyl)-8-azabicyclo[3.2.1]octa-2,6-dien-2-oate.

This reaction is preferably conducted in the presence of a solvent and the solvent is preferably a non-polar solvent. Suitable non-polar solvents for conducting this reaction may be pentane, hexane, and

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benzene. Other suitable non-polar solvents, capable of dissolving the basic reactants may also be employed, with the precise solvent not being critical, as long as it is in fact non-polar.

For details of the hydrogenating, deprotecting and reductive methylation see, the previously incorporated by reference 1991 vol. 56, Journal of Organic Chemistry article. There it is basically described that the catalytic hydrogenation is a process employing a Wilkinson's catalyst and that deprotection occurs with, for example, tertiarybutyl ammonium flouride to give the desired 8-azabicyclo[3.2.1]oct-2-ene at yields as high as 95%. As explained in the earlier referenced article, the composition is purified by silica gel column chromatography.

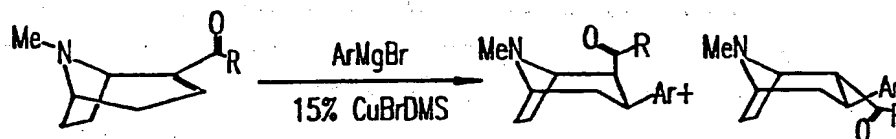
The 8-azabicyclo[3.2.1]oct-2-ene is then used as a starting material for the process of the present invention. It has been found that the 8-azabicyclo[3.2.1]oct-2-ene formula earlier described, can be converted to biologically active cocaine analogs having a wide variety of active analog structures by reacting with a aryl Grignard reagent in the presence of a catalytically effective amount of a copper salt catalyst. The copper salt catalyst may be a copper (I) or copper (II) catalyst.

As previously described, it is preferred that the R group of the 8-azabicyclo[3.2.1]oct-2-ene be C₁ to C₈ alkyl, rather than an oxyalkyl since it is preferred that the two substituent be a ketone substitution rather than an ester substitution. The ketones behave better in the copper catalysed reaction, and as explained later in the biological activity section of

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the specification, should have higher metabolic stability and have equivalent binding site activity. The Grignard addition reaction is run in a suitable non-polar organic solvent, preferably ether or tetrahydrofuran.

The Grignard reagent (ArMgX) may be any suitable aryl magnesium halide. The aryl group may be phenyl, substituted phenyl, C_1 to C_8 alkylaryl, polyaryl such as naphthyl, anthracyl or alkylpolyaryl. Alkyl magnesium halides (C_1 to C_8) may also be used. The "X" moiety represents a halide group and is preferably bromide. The copper salt may be a copper (I) or (II) salt and can be, for example, copper bromide dimethyl sulfide. The amount of the Grignard reagent is preferably an excess of the stoichiometric amount in order to assure completion of the reaction. Suitable high yields are obtained when an excess of up to four-fold of the Grignard reagent is employed. The amount of the copper salt catalyst can be from 5% (molar) to 20% (molar) of the Grignard reagent, and is preferably 15 mole percent of the amount of the Grignard reagent. The reaction to produce the desired ketone is represented by the following equation reaction:



As seen the reaction product is a mixture of two structural isomers, one with the 2-moiety position upwardly (a) and the second with the 2-moiety position downwardly. (b) Those analogs that are most preferred are the analogs wherein R is alkyl and therefore the

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two position moiety is a ketone moiety, and that the structural isomer is with the ketone groups in an up position. These are far more active in binding assays, than the downward structural isomers and in some instances as much as 200 times more active in site-binding.

Certain other process conditions are worthy of mention. The reaction is not temperature critical and may be run at anything from 0°C or lower up to room temperature, or even higher. The reaction is preferably run under an inert gas atmosphere. The reaction is substantially immediate and therefore may be run from a few minutes to as much as twelve hours. Preferably the reaction occurs under stirring in order to assure completeness. After completion the reaction can be quenched with for example HCl/ice, with the desired compound extracted with ether. It may be purified as illustrated in the examples by conventional silica gel chromatography.

The compounds may be administered orally, parenterally or intravenously. The preferred route of administration is oral. The dose levels may be from 4 micrograms per kilogram of body weight up to 50 milligrams/kg of body weight and more typically from 20 micrograms/kg up to 15 mg/kg.

The novel tropane analogs synthesized by the vinylcarbeonoid scheme listed above were tested for their ability to interact with 5-HT and dopamine transporters by two assays: displacement of radioligand binding to transporter sites, and direct inhibition of 5-HT or dopamine uptake in acutely dissociated fetal and adult rat neurons. These assays are known to correlate with transport sites in human

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brain. In binding studies, low concentrations (10-20 pM) of [¹²⁵I]RTI-55, the potent tropane analog recently synthesized by Carroll's group (Boja et al., *European Journal of Pharmacology*, 1991, 184, 329), was used to label dopamine transporters in rat striatal membranes, while [³H]paroxetine (Harbert et al., *European Journal of Pharmacology*, 1985, 118, 107) was used to label 5-HT transporter sites in rat frontal cortex. Up to the present time, 34 analogs have been tested for binding; their potencies in binding and uptake assays are summarized in Table 1 below.

TABLE 1. Tropane analogs: potencies in transporter binding and uptake assays

Drug	R1	R2	R3	¹²⁵ I RT1-55 K _i (nM)	³ H paroxetine K _i (nM)	binding ratio	DA uptake IC ₅₀ (nM)	5-HT uptake IC ₅₀ (nM)	uptake ratio
cocaine	COOPh	COOCH ₃	H	173 ± 19	302 ± 22	1.75	187 ± 11	591 ± 48	3.2
WF-1	Ph-pF	H	COCH ₃	929 ± 160	>10,000
WF-2	Ph	H	COCH ₃	7270 ± 1210	>10,000
WF-3	Ph-pCH ₃	H	COCH ₃	775 ± 57	>10,000
WF-4	Ph-pCH ₃	COCH ₃	H	9.8 ± 0.5	122 ± 22	12.4	13.8 ± 2.6	330 ± 75	24
WF-5	Ph	COCH ₃	H	114 ± 22
WF-6	Ph	H	COOCH ₃	2060 ± 545	>10,000
WF-7	Ph-pF	COCH ₃	H	70.8 ± 13	857 ± 187	12.1	.	.	.
WF-8	1-naphthyl	COCH ₃	H	10.1 ± 2.2	25.6 ± 5.1	2.53	20.7 ± 7.7	4.55 ± 0.040	0.220
WF-9	Ph-pCH ₂ CH ₃	COCH ₃	H	152 ± 24	78.2 ± 22	0.51	1160 ± 266	9.5 ± 5.4	0.0082
WF-10	Ph	COOCH ₃	H	98.8 ± 12.2	1290 ± 51	13.1	.	.	.
WF-11PTT	Ph-pCH ₃	COCH ₂ CH ₃	H	8.2 ± 1.6	131 ± 10	15.9	2.21 ± 1.7	310 ± 104	140
WF-13	Ph-pCH ₃	H	COCH ₂ CH ₃	764 ± 84	>10,000	ND	.	.	.
WF-14	CH ₂ CH ₃	H	COCH ₂ CH ₃	>50,000	>10,000
WF-15	1-naphthyl	H	COCH ₃	97.0 ± 21
WF-16	Ph-pCH ₂ CH ₃	H	COCH ₃	2290 ± 360	>10,000
WF-17	Ph-pCH ₃	H	COCH ₂ (Me) ₂	2130 ± 266	>10,000
WF-18	CH ₂ CH ₃	COCH ₂ CH ₃	H	>50,000
WF-19	cyclohex	COCH ₂ CH ₃	H	4610 ± 492	>10,000
WF-21	PhOCH ₃	H	COCH ₂ CH ₃	1287 ± 322	>10,000
WF-22	PhOCH ₃	COCH ₂ CH ₃	H	814 ± 220	1056 ± 395	1.30	.	.	.
WF-23	2-naphthyl	COCH ₂ CH ₃	H	0.20 ± 0.04	0.63 ± 0.20	3.1	0.027 ± 0.016	0.27 ± 0.13	10.0
WF-23(1)	2-naphthyl	COCH ₂ CH ₃	H	113 ± 26	484 ± 146	4.2	.	.	.
WF-23(2)	2-naphthyl	COCH ₂ CH ₃	H	0.032 ± 0.007	0.127 ± 0.05	3.96	.	.	.
WF-24	2-naphthyl	COCH ₂ CH ₃	H	2.51 ± 0.82	16.4 ± 2.0	6.53	5.9 ± 2.4	.	.
WF-25	Ph	H	COCH ₂ CH ₃	48.3 ± 2.8	1005 ± 112	20.8	.	.	.
WF-26	Ph-p(CH ₃) ₃	COCH ₂ CH ₃	H	2120 ± 630	1771 ± 474	0.84	.	.	.
WF-27	4CH ₃ -1-naph	COCH ₂ CH ₃	H	25.1 ± 0.5	8.99 ± 1.71	0.358	260 ± 98	14.6 ± 10.6	0.056
WF-29	Ph-pF	COCH ₂ CH ₃	H	15.3 ± 2.8
WF-30	1-naphthyl	COCH ₂ CH ₃	H	5.34 ± 1.27	20.9 ± 2.9	3.91	3.66 ± 1.91	5.43 ± 4.63	1.46
WF-31PTT	PhCH(CH ₃) ₂	COCH ₂ CH ₃	H	615 ± 98	54.5 ± 8.9	0.089	5840 ± 2600	46.9 ± 37.1	0.0082
WF-32	2-naphthyl	COCH ₃	H	0.248 ± 0.15	1.06 ± 0.36	4.27	.	.	.
WF-33	6MeO-2naph	COCH ₂ CH ₃	H	0.130 ± 0.036	2.24 ± 0.35
WF-34	6MeO-2naph	H	COCH ₂ CH ₃	54.7 ± 15	206 ± 81	3.76	.	.	.
WF-35	Ph-pPh	COCH ₂ CH ₃	H	2.29 ± 1.1	4.3 ± 0.01	1.88	.	.	.

SUBSTITUTE SHEET (RULE 26)

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In Table 1, the general formula is that depicted in the earlier part of this application, just at the beginning of the heading Detailed Description of the Invention. Code names, as presented, WF-1 through WF-35, are internal names of the assignee and simply stand for "Wake Forest-1" etc. Two analogs have been assigned trivial abbreviations: WF-11 is PTT, and WF-31 is PIT.

The binding affinity of tropane derivatives at the dopamine transporter was the basis of the original patent on drugs for the treatment of cocaine addiction. In the original application, the binding affinities for WF 1-5, 7-9, 11 were reported as background evidence. Since then, a publication with the binding affinities for WF 1-5, 7-9, 11, 13, 18, 19, 22, 23, 25 has appeared (Davies, et al., *European Journal of Pharmacology - Molecular Pharmacology Section*, 1993, 244,93).

Figure 1 compares the displacement of [¹²⁵I]RTI-55 binding by cocaine with four tropane analogs, PTT, PIT, WF-23, and WF-33. This leads to information on how these compounds bind to the dopamine transporter. These data showed that the two 2-naphthyl analogs, WF-23 and WF-33, were the most potent of these compounds, followed by PTT. PIT, in contrast, was less potent in displacing [¹²⁵I]RTI-55 than cocaine. Comparison of IC₅₀ values (Table 1) showed that WF-23 and WF-33 were 900 to 1300 times, while PTT was 20 times, more potent than cocaine in binding to dopamine transporters. In contrast, PIT was 2.5 times less potent than cocaine at dopamine transporters.

Figure 2 shows how the selectivities of these analogs were determined in [³H]paroxetine binding

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experiments. This leads to information on how these compounds bind to the 5-HT transporter. Again, the 2-naphthyl analogs, WF-23 and WF-33, were the most potent compounds in displacing [^3H]paroxetine binding, as they were vs. [^{125}I]RTI-55 binding (Fig. 1). However, whereas these analogs were equipotent in displacing [^{125}I]RTI-55 binding, WF-33 was 4 times less potent than WF-23 in displacing [^3H]paroxetine binding. Furthermore, the two phenyl analogs, PTT and PIT, exchanged places in displacing [^3H]paroxetine compared to [^{125}I]RTI-55; PIT was significantly more potent in displacing [^3H]paroxetine than cocaine, while PTT was approximately equipotent with cocaine in displacing [^3H]paroxetine binding (Fig. 2). IC_{50} values (Table 1) showed that WF-23 and WF-33 were 480 times and 140 times, respectively, more potent than cocaine at 5-HT transporter sites, while PIT was 8 times more potent than cocaine and PTT was twice as potent as cocaine. Table 1 shows the potency ratios of all analogs in binding assays dopamine and 5-HT transporters (higher numbers demonstrate relatively greater dopamine transporter potency). These data suggested that PIT was relatively selective for 5-HT transporters, while PTT and PIT were relatively more selective for dopamine transporters. In contrast, WF-23 was like cocaine, with little selectivity between the two transporters. However, it was 500-800 times more potent than cocaine at both transporters.

The synthetic scheme that was used to produce tropane analogs from vinylcarbenoid precursors generated racemic compounds; therefore, all the binding studies discussed above were conducted with racemic compounds. When WF-23 was separated into two

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stereoisomers by a chiral HPLC column, the active isomer displayed an estimated IC_{50} value of 0.03 nM vs. [125 I]RTI-55, while the inactive isomer demonstrated an IC_{50} value of 113 nM (see values for WF-23(1) and WF-23(2) in Table 1). These results not only demonstrated that stereoisomers can be separated by the chiral HPLC, but also shows that the active isomer is extremely potent. The active isomer of WF-23 was also potent vs. [3 H]paroxetine binding, and the selectivity of the active and inactive isomers were the same as the racemic mixture of WF-23. Generally those isomers with R_2 in the up position were more active, and as well, those compounds where R_2 was a ketone were more active.

Uptake studies have been conducted on several selected analogs to confirm the results of the binding studies. These experiments utilized dissociated cells from fetal and adult rat brain, using the striatum for dopamine uptake assays, and the frontal cortex for 5-HT uptake assays. Fig. 3 shows the inhibition of [3 H]dopamine uptake into striatal cells by cocaine and selected tropane analogs. These results were comparable to the binding assays: while WF-23 was the most potent analog in inhibiting dopamine uptake followed by WF-11, WF-31 was considerably less potent in blocking [3 H]dopamine uptake. Experiments with [3 H]5-HT uptake in cortical cells (Fig. 4) also supported the results of the binding assays, showing that WF-23 and WF-31 were both significantly more potent than cocaine in blocking [3 H]5-HT uptake. Thus the uptake assays confirmed the selectivities of these tropane analogs as determined in binding assays. For example, WF-11 was 140 times more potent in inhibiting

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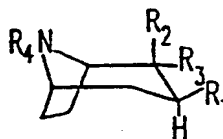
dopamine uptake than 5-HT uptake, while WF-31 was 120 times more potent in inhibiting 5-HT uptake than dopamine uptake, both somewhat greater than the ratios determined by binding studies (Table 1). In contrast, WF-23, whether assayed as a racemic mixture or as its active stereoisomer, provided a dopamine: 5-HT ratio of only 3-4 regardless of the assay used.

In addition to the isopropylphenyl derivative WF-31, it is also clear that the ethylphenyl derivative WF-9, and the 1-(4-methylnaphthyl) derivative WF-27 also display considerable selectivity towards the 5-HT transporter in terms of binding and inhibition. The common feature of these derivatives is that they contain a functionality that may lie to some extent in the perpendicular plane to the aromatic ring. This structural variation leads to a novel type of biological activity for compounds in the tropane series with potential for the development of a novel class of antidepressant drugs. The general activity of the group of analogs at monoamine transporter sites demonstrates that they have potential for the treatment of other diseases associated with monoamine imbalances such as Parkinson's Disease, attention-deficit hyperactivity disorder, and obesity.

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WHAT IS CLAIMED IS:

1. A method of treating mammals to selectively block 5-HT uptake, said method comprising administering a small but effective amount of a 3-aryltropane derivative of the formula:



and structural isomers thereof, wherein R_1 is an aromatic ring moiety selected from the group consisting of 1-naphthyl, 2-naphthyl, phenyl, C_1 to C_8 alkylaryl, and indole; and R_2 and R_3 may be the same or different and are selected from the group consisting of hydrogen, C_1 to C_8 ketones, esters, phosphonates, sulfones, cyano, oxazole and imidazole, with only one of R_2 and R_3 being hydrogen at any one time. R_4 is methyl, hydrogen or lower alkyl.

2. The method of claim 1 where R_2 is a ketone.
3. The method of claim 1 wherein R_2 is the isomer with R_2 in the up position.
4. A method of treating mammals to selectively block 5-HT uptake, said method comprising administering a small but treatment effective amount of a 3-aryltropane derivative of the formula:

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and structural isomers thereof wherein R equals C₁ to C₈ alkyl and Ar is an aromatic ring moiety, to said mammals.

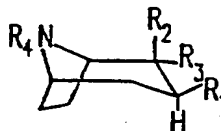
5. The method of claim 1 wherein the mammal is the human species.

6. The method of claim 2 wherein the administration is by a method selected from the group of oral, intravenous, and parenteral.

7. The method of claim 2 wherein the dosage is at a level of from 1 micrograms/Kg to 50 milligrams/Kg.

8. The method of claim 2 wherein the dosage is oral at a level of from 20 micrograms/Kg to 15 mg/Kg.

9. A method of treating mammals to selectively block Dopamine uptake, said method comprising administering a small but effective amount of a 3-aryltropane derivative of the formula:



and structural isomers thereof, wherein R₁ is an aromatic ring moiety selected from the group consisting of 1-naphthyl, 2-naphthyl, phenyl, C₁ to C₈ alkylaryl, and indole; and R₂ and R₃ may be the same or different and are selected from the group consisting of hydrogen, C₁ to C₈ ketones, with only one of R₂ and

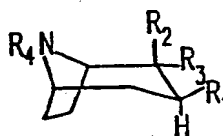
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R_3 being hydrogen at any one time. R_4 is methyl, hydrogen or lower alkyl.

10. The method of claim 9 where R_2 is a ketone.

11. The method of claim 9 wherein R_2 is the isomer with R_2 in the up position.

12. A method of treating mammals to selectively block 5-HT uptake, said method comprising administering a small but treatment effective amount of a 3-aryltropane derivative of the formula:



and structural isomers thereof wherein R equals C_1 to C_8 alkyl and Ar is an aromatic ring moiety, to said mammals.

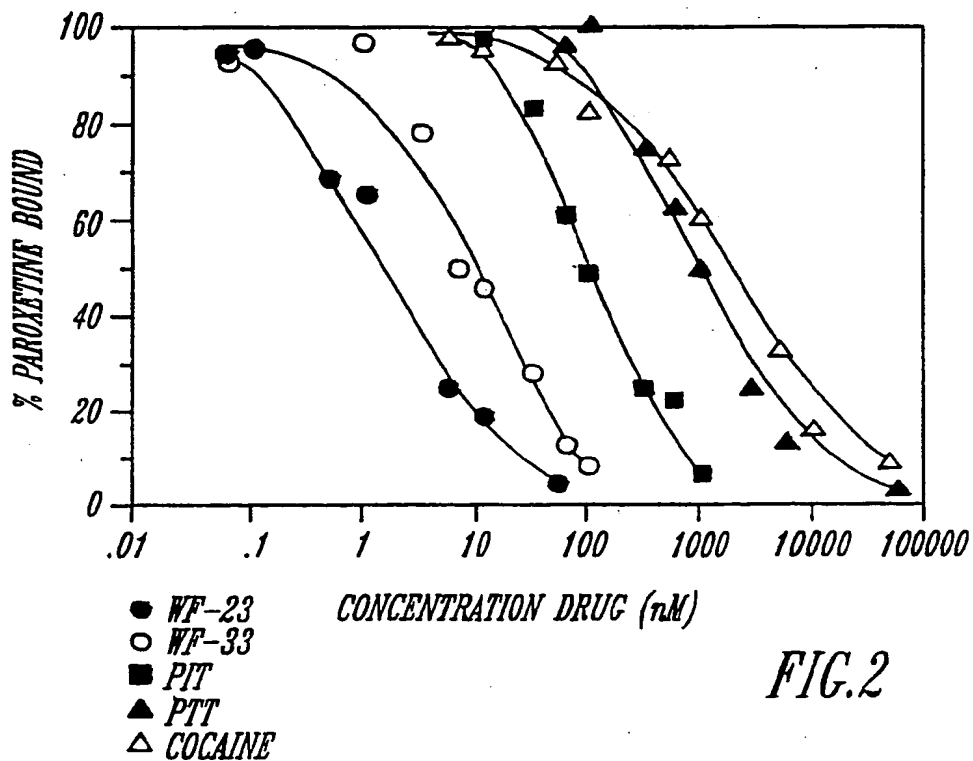
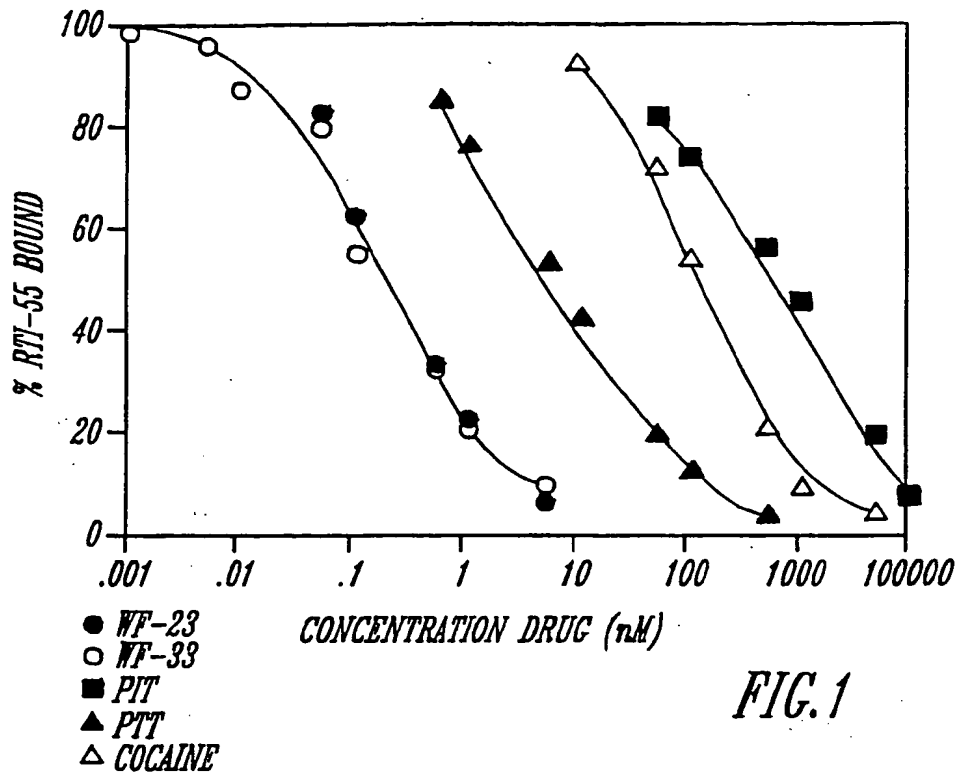
13. The method of claim 9 wherein the mammal is the human species.

14. The method of claim 10 wherein the administration is by a method selected from the group of oral, intravenous, and parenteral.

15. The method of claim 10 wherein the dosage is at a level of from 1 micrograms/Kg to 50 milligrams/Kg.

16. The method of claim 10 wherein the dosage is oral at a level of from 20 micrograms/Kg to 15 mg/Kg.

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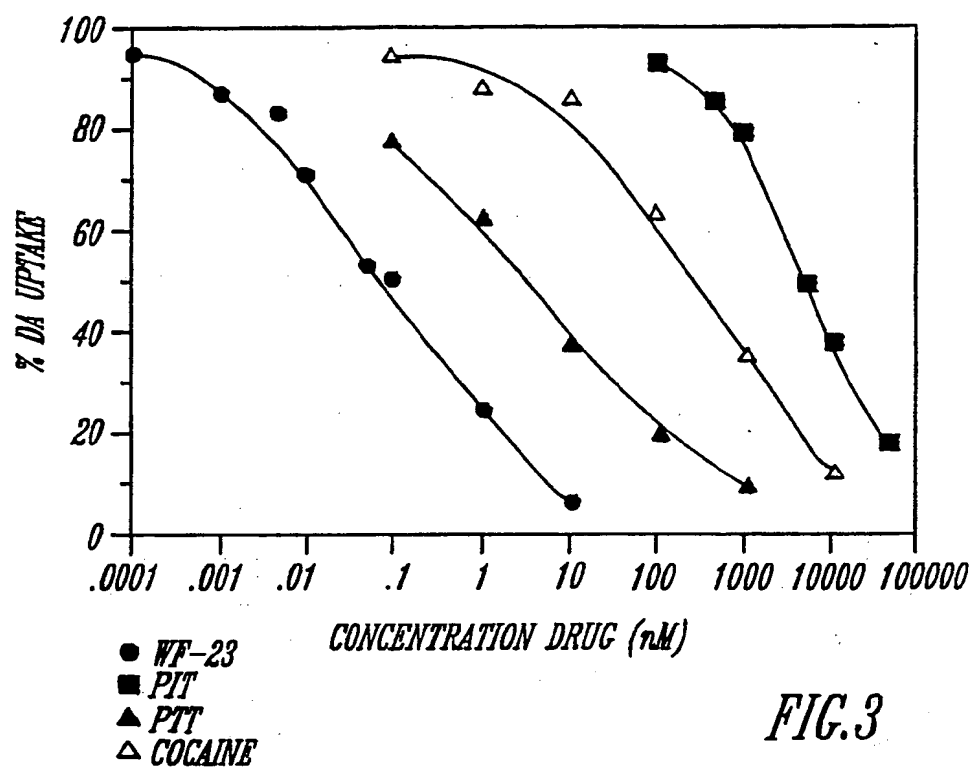


FIG. 3

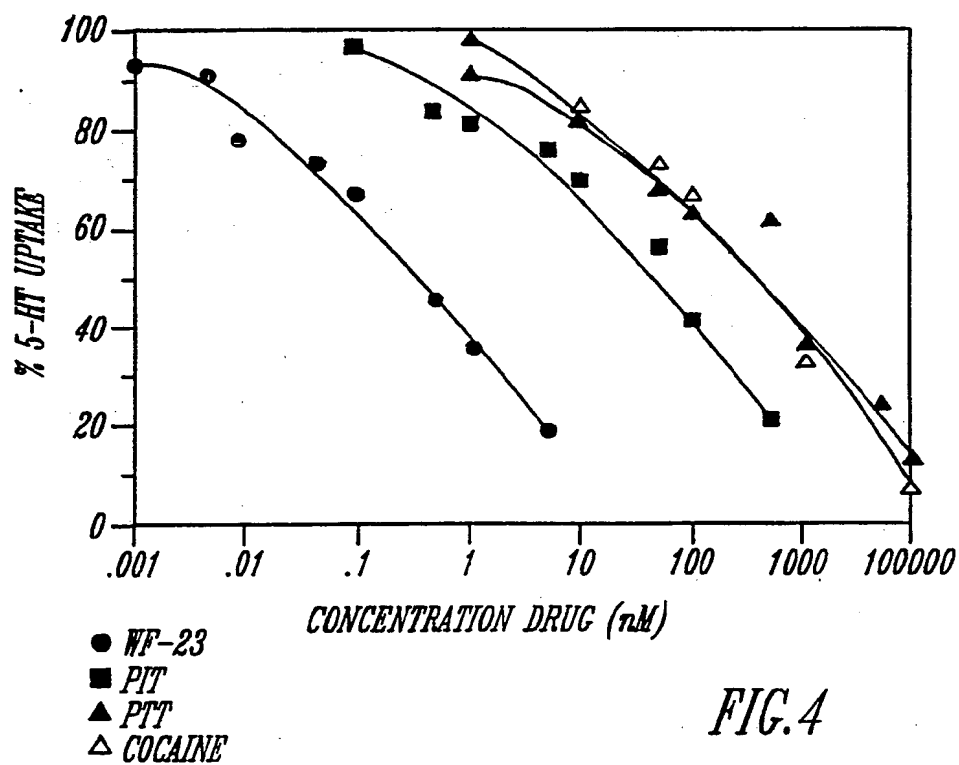


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03661

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/44, 31/675
US CL :514/304, 299, 80
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/304, 299, 80

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS,CAS- CLAIMED COMPOUNDS AND SEROTONIN AND DOPAMINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIFE SCIENCES, VOLUME 46, NO. 9, ISSUED 1990, M.C. RITZ ET AL., "COCAINE INHIBITION OF LIGAND BINDING AT DOPAMINE, NOREPINEPHRINE AND SEROTONIN TRANSPORTERS: A STRUCTURE-ACTIVITY STUDY", PAGES 635-645, SEE ESPECIALLY THE ABSTRACT AND PAGES 638-9.	1-3
A	US, A, 3,813,404 (CLARKE ET AL.) 28 MAY 1974, SEE COLUMN 1, LINES 22-51 AND COLUMN 3, LINES 5-13 AND 43-52.	1-3, 5-11 AND 13-16
A	EUROPEAN JOURNAL OF PHARMACOLOGY- MOL. PHARM. SECT., VOL. 244, ISSUED 1993, H.M.L. DAVIES ET AL., "NOVEL 2-SUBSTITUTED COCAINE ANALOGS: BINDING PROPERTIES AT DOPAMINE TRANSPORT SITES IN RAT STRIATUM", PAGES 93-97.	1-3, 5-11 AND 13-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

23 JUNE 1994

Date of mailing of the international search report

AUG 01 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03661

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 4 AND 12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

CLAIM 4 LACKS A STRUCTURAL FORMULA FOR THE INTENDED CHEMICAL COMPOUNDS. CLAIM 12 FAILS TO DEFINE THE SUBSTITUENTS R1-R4.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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